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Enhancement of Chromosomal Damage by Arsenic: Implications for Mechanism

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Arsenic is a naturally occurring metalloid that has been associated with increased incidence of human cancer in certain highly exposed populations. Arsenic is released to the environment by natural means such as solubilization from geologic formations into water supplies. It is also released to occupational and community environments by such activities as nonferrous ore smelting and combustion of fuels containing arsenic. Several lines of evidence indicate that arsenic acts indirectly with other agents to ultimately enhance specific genotoxic effects that may lead to carcinogenesis. Work described here indicates that arsenite specifically potentiates chromosomal aberrations induced by a DNA crosslinking agent, 1,3-butadiene diepoxide, but does not effect the induction of sister chromatid exchanges under the same treatment conditions. It is proposed that the specific co-clastogenic effects of arsenite seen here may be mediated by its interference with DNA repair activities. Further understanding of the mechanism by which arsenic interacts with other environmental agents will result in more accurate estimates of risk from exposure to arsenic.

Background

Arsenic is naturally occurring in various geological formations and is released to the environment by solubilization into groundwater supplies (*1*). Release can also occur into community and occupational environments by such activities as nonferrous ore smelting and production of electric power by burning coal containing arsenic. When such coal is combusted, minute quantities of arsenic are emitted to the atmosphere depending on the arsenic content of the fuel. Arsenic content in U.S. bituminous coal may vary from about 0.02 μg/g up to 360 μg/g (*2*). Health risk estimates for current coal-fired utility arsenic emission levels have been derived using the inhalation unit risk estimates developed by the U.S. Enviromental Protection Agency (EPA) (*3*). "Maximum individual lifetime" risk for cancer from arsenic from a modeled coal-fired power plant is estimated by EPA to be 1×10^{-5}.

Arsenic exists principally in two valence states: As^{3+} (arsenite) or As^{5+} (arsenate). Arsenite is considerably more acutely toxic than is arsenate, but the relationship of valence state to the potential induction of carcinogenesis is unknown. Arsenic also exists in organic forms and is often found in relatively high amounts in this form in fish as arsenobetaine.

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Arsenic is a well-known toxin and is considered a human carcinogen based on epidemiological evidence (*4,5*). Studies of occupational exposure to high air concentrations of arsenic in copper smelters (*6–11*) and community exposure to significant arsenic levels in drinking water (*12*) have indicated an increased risk of lung and skin cancer, respectively. Follow-up studies of exposure to arsenic via drinking water in Taiwan have implied that increases in cancer at other internal sites such as bladder and kidney may have occurred as well (*13–15*). Populations studied in both occupational and community settings were simultaneously exposed to complex mixtures of other compounds and trace elements; there is also some evidence that nutritional and lifestyle factors such as smoking may have interacted with arsenic to produce a synergistic response (*16,17*).

In animal studies, arsenical compounds alone have not yielded a directly tumorigenic response (*5*). However, a mixture of arsenic trioxide, sulfuric acid, and particulates did induce pulmonary carcinomas in hamsters when administered by intratracheal instillation (*18*). Results of animal studies provide support for the rationale that arsenic acts in concert with other agents to alter or enhance biological effects potentially including steps in progression to carcinogenesis (*19*).

A number of hypotheses have been described concerning the possible mechanism of action of arsenic in cancer. Unlike most initiating chemicals, arsenic is inactive or extremely weak in its ability to directly induce gene mutations; however, it does cause gene amplification and may act as a tumor promoter (*20,21*). It has been suggested that arsenical compounds may interact antagonistically or syn-

ergistically with other genotoxic exposures in humans (*22*). Epidemiological studies have indicated a synergistic interaction between arsenic exposure and cigarette smoking in smelter workers in the induction of lung cancer (*17,23*) and in the production of chromosomal aberrations in smelter workers (*24*). Arsenite has been shown to enhance the mutagenicity of UV treatment in bacterial cells (*25*) and to inhibit DNA ligase activity in mammalian cells (*26*). The induction of chromosomal aberrations in human cells by DNA crosslinking agents combined with UV light was observed to be synergistically enhanced by arsenite (*27–29*).

To further explore the possible potentiation of genotoxic damage by arsenite, experiments were undertaken to examine effects of arsenite exposure on the induction of chromosomal aberrations and sister chromatid exchanges by the DNA crosslinking agent 1,3-butadiene diepoxide [DEB (*30*)].

Specificity of Arsenite in Enhancing Chromosomal Effects

Methods

Peripheral lymphocytes from three subjects were cultured in RPMI 1640 tissue culture medium with supplements. Subject 1 was DEB-sensitive to sister chromatid exchange (SCE) induction, whereas subjects 2 and 3 were resistant to such induction as previously described (*31,32*). At 24 hr of culture, lymphocytes were treated with 0.5, 1.0, 1.5, and 2.0 μM sodium arsenite or 6 μM DEB. In a third series of experiments, DEB and arsenite were combined to treat cultured cells. In this protocol, DEB was added first, followed immediately by arsenite. Immediately after treatment, 50 μM bromodeoxyuridine was added. Cells were cultured for 72 hr at 37.5°C and treated with colcemid 2 hr before harvest. Standard chromosome preparations were differentially stained using a modification of the fluroescence-plus-Giemsa technique (*33*). For each subject, 100 first-division cells were scored per treatment for aberration frequencies. Aberrations scored were chromosome-type aberrations and chromatid and isochromatid deletions and chromatid exchanges. To estimate SCE frequencies, 30 second-division cells were scored per point; replication indexes were determined. Linear regression was applied to ascertain trends in aberration rates or SCE frequencies. Pairwise differences between observed and expected frequencies of chromosomal aberrations were assessed by the chi-square test; the student's *t*-test was used to test for differences in mean SCE frequencies. Bonferroni's method to correct for multiple comparisons was used to adjust the α level for significance ($p < 0.05$).

Results and Discussion

The yields of total chromosomal aberrations in lymphocytes exposed to both arsenite and DEB together were markedly increased above the levels expected if effects of the two agents had been simply additive (Fig. 1). For the DEB-sensitive subject (Fig. 1*a*), more than 300 chromosome aberrations were induced per 100 cells with the combined treatment at 2 μM sodium arsenite – an approximately 10-fold increase above expected.

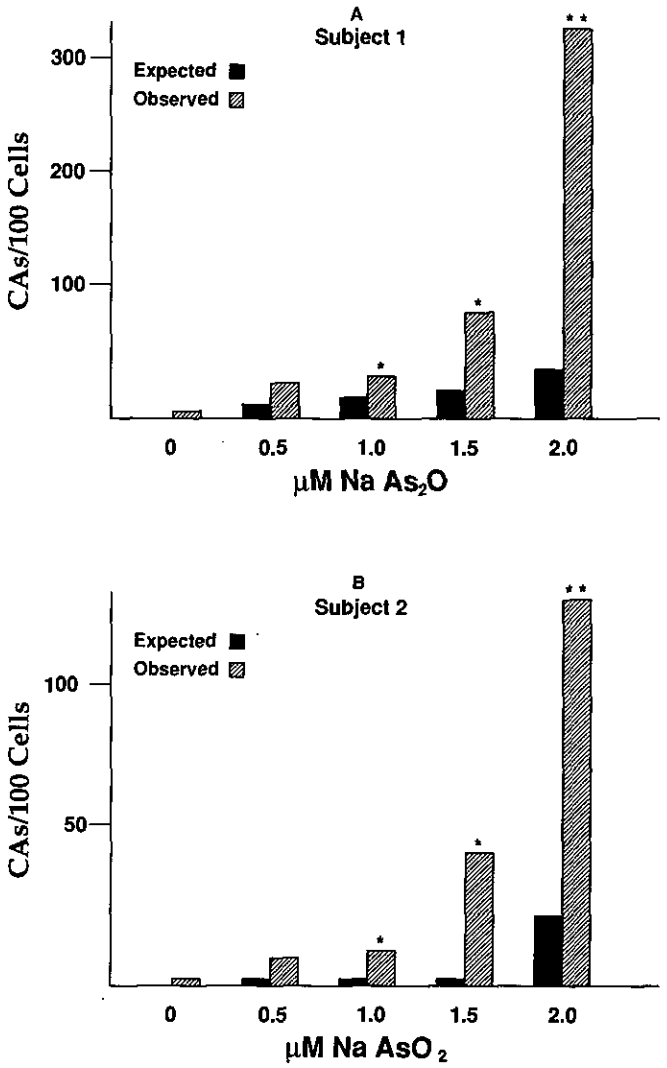

FIGURE 1. The effects of combined treatment with sodium arsenite ($NaAsO_2$) and 6 μM 1,3-butadiene diepoxide (DEB) to produce enhanced yields of total chromosomal aberrations. (*A*) Results from subject 1, whose lymphocytes were relatively sensitive to induction of sister chromatid exchanges (SCEs) by DEB. (*B*) Results from subject 2, whose lymphocytes were relatively resistant to DEB-induced SCEs. (*) Observed frequency significantly different from expected ($p < 0.05$; χ^2 test); (**) observed frequency significantly different from expected ($p < 0.001$; χ^2 test).

As seen in Figure 1*b*, results of the combined treatment for subject 2, whose cells were relatively resistant to SCE induction by DEB, showed similar results. In this instance, however, about a 5-fold increase in the number of chromosome aberrations was observed relative to the number expected. Cells from subject 3 showed similar results.

In contrast, as shown in Figure 2, the yields of induced SCEs are additive for the combined DEB plus arsenite treatments, thus the expected and observed frequencies of SCEs are not significantly different. There were no sub-

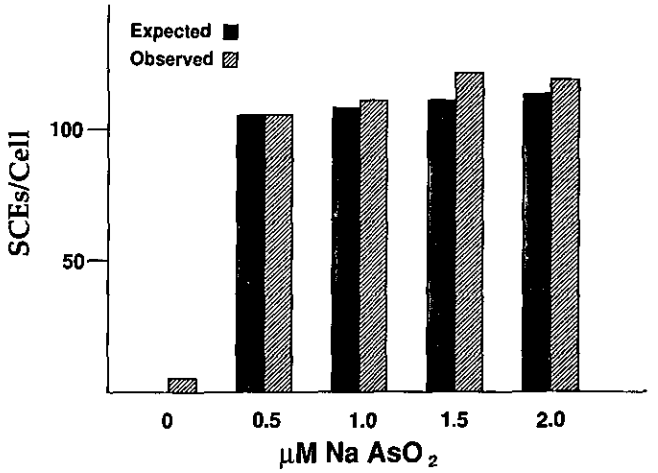

FIGURE 2. The effect of combined treatment with sodium arsenite ($NaAsO_2$) and 6 μM 1,3-butadiene diepoxide on induction of sister chromatid exchanges (SCEs) in subject 1. No significant differences between expected and observed SCE frequencies were noted for any of the three subjects. All values have been corrected by subtracting out the appropriate control (background) SCE frequency.

stantial differences among the three subjects with regard to this response.

These results show that arsenite acts synergistically with DEB in the induction of chromosomal aberrations but does not affect the yield of DEB-induced SCEs. Interestingly, neither the DEB-sensitive nor DEB-resistant subjects showed a synergistic effect with arsenite in the induction of DEB-induced SCEs. In contrast, the interaction of arsenite with DEB in the induction of chromosomal aberrations was found to be greatest in lymphocytes from the subject sensitive to SCE induction by DEB.

Although it has been recognized that arsenite reacts with protein sulfhydryl groups, it has now been shown that arsenite may be highly selective in reacting with only a small number of closely spaced dithiol groups in proteins (*34,35*). Such dithiol groups are relatively common in DNA-associated protein molecules including DNA repair proteins (*36*). Specifically, the activity of DNA ligase, a mammalian DNA repair protein, has been shown to be inhibited by arsenite (*26*).

Conclusion

Arsenic is a ubiquitous metalloid known to be associated at relatively high exposure concentrations with an increased risk for certain cancers in humans. Arsenic does not appear to act directly as an initiator, but rather indirectly by as yet unknown mechanism(s) to enhance the effect of other genotoxic agents. Understanding of such mechanisms has implications for the shape of the dose-response curve. Recent studies, including the study summarized here, provide further evidence for arsenite as a co-clastogen. This study also suggests a specific interaction of arsenite with the induction or repair of DNA damage produced by DEB that leads to chromosomal aberrations but not to SCEs. Further studies are necessary to identify DNA repair proteins containing dithiol groups sensitive to low concentrations of arsenic. This work and other studies previously discussed support the proposal that arsenic exposure in combination with other DNA damaging agents leads to a specific enhancement of effects related to genotoxicity that may be important in development of cancer.

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